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Chemical Composition and *In Vitro* Protein Digestibility of Duckweed and Feed Ingredients

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Abstract

Duckweed is an aquatic floating plant with the potential to be used as animal feed due to its richness in protein, amino acids, starch, vitamin and minerals. Furthermore, duckweed has a high growth rate and reproducibility. Therefore, this research aimed to determine the chemical composition and *in vitro* protein digestibility of duckweed (*Lemna*, *Wolffia* and *Spirodela*) in comparison to other feed ingredients. The chemical composition was determined through proximate analysis including dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), ash and nitrogen free extract. The *in vitro* protein digestibility procedure simulates conditions similar to those in the swine digestive tract using synthetic enzymes to control pH, temperature and digestion time. In the results of chemical composition, *Lemna* and *Wolffia* were classified as protein sources, with high CP content (221.87 and 442.65 g/kg DM, respectively) and low CF content (63.30 and 109.11 g/kg DM), whereas *Spirodela* had low CP content (179.64 g/kg DM) and CF content (170.57 g/kg DM). The EE content in duckweed amounted to 16.74, 46.23 and 52.47 g/kg DM for *Wolffia*, *Spirodela* and *Lemna*, respectively. Additionally, high ash content was observed in *Spirodela* (212.40 g/kg DM), *Wolffia* (149.34 g/kg DM) and *Lemna* (142.06). For standardized ileal digestibility (SID_{CP}), the SID_{CP} of all feed ingredients were within the range of 32 to 96 %. With regard to duckweed, *Lemna* (72 %) showed significantly higher ($p < 0.05$) SID_{CP} compared to *Wolffia* (69 %) and *Spirodela* (39 %) due to the low contents of CF and ash. Based on nutritional values, *Lemna* and *Wolffia* were grouped with corn DDGS and brewer's grain, indicating their potential as animal feed for sustainable livestock production.

Keywords: Chemical composition, Duckweed, Feed ingredient, *In vitro* digestibility, Protein

Introduction

The world's population is estimated to increase from 7.70 billion in 2019 to 9.70 billion in 2050, resulting in a greater demand for animal products [1]. Feed cost is a critical factor, accounting for at least 60 % of the total production costs in poultry [2]. In animal diets, the most common protein sources include soybean products, fishmeal, milk products and rapeseed meal [3,4]. Meanwhile, corn and broken rice are commonly used as energy sources [5]. Due to the high price of these products, various by-products from agriculture and the food industry such as rice bran, palm kernel meal, coconut meal, distillers dries grains with soluble (DDGS) and feather meal have been introduced as supplements in animal feeds to reduce feed costs without affecting growth performance [6,7]. Furthermore, alternative feed ingredients are needed to meet the increasing demand for sustainable animal production in the near future.

Duckweed is a tiny aquatic floating plant in the Lemnacae family [8]. There are 37 species distributed across 5 genera: *Lemna*, *Wolffia*, *Spirodela*, *Landoitia* and *Wolffiella* [9]. Duckweed can grow in water resources worldwide, except in dry and frozen area [10]. It has the potential to be utilized as a feed ingredient due to its rapid growth, doubling in amount within only 2 to 7 days [11,12]. The chemical composition in duckweed includes 252.00 to 365.00 g/kg dry matter (DM) for crude protein (CP), 45.00 to 66.00 g/kg DM for ether extract (EE), 88.00 to 110.00 g/kg DM for crude fiber (CF) and 40.00 to 436.00 g/kg DM for carbohydrates. Additionally, it contains high contents of essential amino acids such as lysine (33.70 to 42.60 g/kg DM), methionine (8.30 to 10.70 g/kg DM) and threonine (25.50 to 34.50 g/kg DM) [13,14]. Duckweed is also an abundant source of sugars, vitamins and minerals [15] as well as pigments such as beta-carotene and xanthophyll, which can be supplemented in the diets of poultry and aquatic animals [16]. In a recent report by Demann *et al*. [17], it was found that the *in vitro* digestibility of *Lemna* and *Spirodela* in ranged from 61.90 to 78.60 %, and *Lemna obscura* can be supplemented in broiler chicken diets up to 25 % due to its high amino acid content and high standardize ileal digestibility (SID) of amino acids. Furthermore, duckweed can replace up to 20 % of soybean meal in laying hen diets, leading to an increase in yolk color score without a negative effect on egg quality [18]. Similarly, replacing sesame oil cake with 6 % duckweed in broiler chicken diets has been shown to improve body weight, feed intake and feed conversion ratio [19]. For pigs, the use of duckweed at levels of 40 or 60 % in nursery pig diets can improve palatability, feed intake and average daily gains, which are higher than those in nursery pigs fed soybean meal as the only source of protein [20]. Besides, the SID of amino acids of *Lemna* protein concentration in nursery pigs was comparable to that of fish meal [21]. This information indicates that duckweed can be used as a valuable protein source in diets for livestock production.

In feed formulation, *in vitro* digestibility assays have been developed to estimate digestibility of CP and amino acids (AA) in feed ingredients for monogastric animals [22-24]. The 2-step method using pepsin and pancreatic enzymes is generally used to simulate the digestive processes in the stomach and small intestine [22], and there were strong linear relationships between *in vitro* and *in vivo* digestibility in the assay feed ingredients [24]. This technique is an alternative to *in vivo* digestibility due to its cost-effective and less time-consuming [25]. Therefore, the objective of this research is to determine the chemical composition and *in vitro* protein digestibility of duckweeds in comparison to other feed ingredients.

Materials and methods

Duckweed management and collection

Three duckweed genera, including *Lemna*, *Wolffia* and *Spirodela*, were obtained from the Advance Greenfarm Co., Ltd. (Nakhon Pathom, Thailand). They were cultivated in the pond with PVC-coated fabric and supplemented with fertilizers. The duckweed was harvested every 3 or 4 days using a nylon net, and then sun-dried for 2 or 3 days (approximately 6 to 8 % moisture). The dried duckweed was stored at room temperature until chemical analysis.

Sample preparation and chemical composition

Duckweed, including *Lemna*, *Wolffia* and *Spirodela*, was compared to other feed ingredients such as feather meal, fishmeal, soybean meal (47 % CP), soybean meal (44 % CP), rapeseed meal, full-fat soybean, skim milk, brewer's grain, corn DDGS, broken rice, corn, rice bran, coconut meal, palm kernel meal and soybean hull. The samples were ground using an Ultra centrifugal mill (CT 293, FOSS Co., Ltd., Hillerod, Denmark) and then sieved through a 0.5 mm mesh screen for proximate analysis. The chemical composition was analyzed according to the official standard methods [26]. The DM was determined using a hot air oven at 100 to 105 °C for 4 to 6 h. The CP content was determined using the Kjeldahl method, and then calculated by multiplying the nitrogen content by a factor of 6.25. The ether extract (EE) was determined using petroleum ether, whereas crude fiber (CF) was determined according to the Van Soest method. The crude ash was obtained by burning in a Muffle furnace at 600 °C for 6 h. The nitrogen-free extract (NFE) was calculated as 100 minus by sum of CP, EE, CF and crude ash.

In vitro digestibility techniques

In vitro digestibility of CP was determined as described by Jezierny *et al.* [24]. Each sample (0.5 g) was placed in 250 mL conical flask. Subsequently, 25 mL of a 0.1 g/mL phosphate buffer A (pH 6.0) was added to the conical flask and mixed. Thereafter, 10 mL of a 0.2 mol/L HCl solution was added, and the pH was adjusted to 2 using either a 5 mol/L HCl or a 5 mol/L NaOH solution to simulate conditions similar to those in the animal’s stomach. The next step involved adding 1 mL of a freshly prepared 0.01 g/mL pepsin solution (0.7 FIP-U/mg, EC 3.4.23.1, Merck KGaA, Darmstadt, Germany) to the conical flask and mixing. Additionally, 0.1 mL of a chloramphenicol solution was added to prevent bacterial contamination. The conical flask was covered with aluminum foil and placed in a shaking water bath (FWS-30, Faithful®, Huanghua Faithful Instrument Co. Ltd., Hebei, China) set at 40 ± 1 °C, and shaken continuously for 6 h. The conical flask was taken out from the shaking water bath. Afterward, 5 mL of a 0.6 mol/L NaOH and 10 mL of a 0.2 g/mL phosphate buffer B (pH 6.8) were added to the conical flask and mixed. The pH was adjusted to 6.8 using either a 5 mol/L HCl or a 5 mol/L NaOH to stimulate conditions similar to those in the animal’s small intestine. To this mixture, 1 mL of a freshly prepared 0.05 g/mL pancreatin solution (P1750-100G, Sigma-Aldrich®, Merck KGaA, Darmstadt, Germany) was added to the conical flask and mixed. Thereafter, the conical flask was covered with aluminum foil and placed in a shaking water bath set at 40 ± 1 °C, and shaken continuously for 18 h. Afterward, 5 mL of a 20 % sulphosalicylic acid solution was added, and the temperature was adjusted to 20 ± 1 °C. Subsequently, each conical flask was sealed with aluminum foil and placed in a shaking water bath set at 20 ± 1 °C for 30 min. The undigested residues were filtered into a fiber bag with a particle size 38 µm (10-0127 FibreBags-ADF, C. Gerhardt GmbH & Co. KG, Gerhardt, Germany) using a glass funnel. The undigested residue was rinsed with a 1 % sulphosalicylic acid solution and 10 mL of 96 % ethanol, and then underwent 2 consecutive steeping in acetone, each lasting 3 min. The undigested residues were then dried in a hot air oven at 100 to 105 °C for 4 to 6 h. Following the drying process, the nitrogen content was determined using the Kjeldahl method.

Calculations

In vitro digestibility of CP (TD_{CP}) was calculated using the equation proposed by Jezierny *et al.* [24] as follows:

$$\text{True digestibility (\%)} = \left(\frac{\text{CP in the sample} - \text{CP in the undigested residues}}{\text{CP in the sample}}\right) \times 100$$

The true digestible value of CP (dTD_{CP}) was calculated by multiplying the CP content (g/kg DM) of each respective feed ingredient based on the equation as follows:

$$dTD_{CP}\ (g/kg\ DM) = \frac{CP \times DN}{1,000}$$

where DN was *in vitro* digested nitrogen (g/kg DM). The standardized ileal digestibility (SID) of CP (SID_{CP}) was obtained by correcting for specific endogenous loss of CP (SEL_{CP}). The SEL_{CP} was calculated using the following equation:

$$SEL_{CP}\ (g/kg\ DM) = 0.066 \times UDM$$

where UDM was *in vitro* undigested DM (g/kg DM). The digestible SID of CP ($dSID_{CP}$) was calculated using the following equation:

$$dSID_{CP}\ (g/kg\ DM) = CP_{TD} - SEL_{CP}$$

The SID_{CP} was calculated using the following equation:

$$SID_{CP}\ (\%) = \left(\frac{dSID_{CP}}{CP}\right) \times 100$$

Statistical analysis

The data was statistically analyzed using the ANOVA procedure of the SAS software package (SAS Inst., Inc., Cary, NC, USA). The significant differences between treatments were set at $\alpha = 0.05$ using Duncan's new multiple range test. The *K*-means clustering method was implemented using the R program Version 4.3.1 [27] to group duckweed with all feed ingredients based on chemical composition and *in vitro* protein digestibility [28].

Results and discussion

Chemical composition of duckweed and feed ingredients

The chemical composition of duckweed and other feed ingredients are shown in **Table 1**. Protein sources were classified with a minimum CP of 200.00 g/kg DM and CF less than 180.00 g/kg DM, whereas energy sources were classified with CP less than 200.00 g/kg DM and CF less than 180.00 g/kg DM [29]. With this regard, the protein sources consisted of feather meal (903.87 and 7.03 g/kg DM for CP and CF, respectively), fishmeal (651.27 and 2.90 g/kg DM for CP and CF, respectively), soybean meal (47 % CP; 522.28 and 28.02 g/kg DM for CP and CF, respectively), soybean meal (44 % CP; 467.75 and 54.47 g/kg DM for CP and CF, respectively), rapeseed meal (407.60 and 79.87 g/kg DM for CP and CF, respectively), full-fat soybean (385.97 and 40.70 g/kg DM for CP and CF, respectively), skim milk (328.57 for CP and no CF content), brewer's grain (314.36 and 116.25 g/kg DM for CP and CF, respectively) and corn DDGS (307.10 and 82.09 g/kg DM for CP and CF, respectively). In addition, energy sources consisted of broken rice (95.19 and 14.22 g/kg DM for CP and CF, respectively), corn (90.02 and 17.16 g/kg DM for CP and CF, respectively), rice bran (161.36 and 92.31 g/kg DM for CP and CF, respectively) and coconut meal (138.52 and 178.15 g/kg DM for CP and CF, respectively). Furthermore, other groups included palm kernel meal (168.01 and 181.18 g/kg DM for CP and CF, respectively) and soybean hull (122.94 and 449.55 g/kg DM for CP and CF, respectively). For duckweed, CP content was higher in *Wolffia* (442.65 g/kg DM) than *Lemna* (367.81 g/kg DM) and *Spirodela* (179.64 g/kg DM), whereas CF content was higher in *Spirodela* (170.57 g/kg DM) than *Lemna* (109.11 g/kg DM) and *Wolffia* (63.30 g/kg DM).

Table 1 Chemical composition of duckweed and feed ingredients (g/kg DM).

Item	Dry matter	Crude protein	Ether extract	Crude fiber	Ash	NFE
Protein sources						
Feather meal	931.97 ± 0.37	903.87 ± 2.10	83.79 ± 3.25	7.03 ± 1.58	12.50 ± 0.67	nd
Fishmeal	931.16 ± 1.42	651.27 ± 1.83	61.41 ± 0.72	2.90 ± 0.27	265.24 ± 3.02	20.26 ± 5.12
Soybean meal 47 % CP	903.52 ± 5.72	522.28 ± 1.18	23.64 ± 1.79	28.02 ± 0.90	76.78 ± 1.64	347.92 ± 0.27
Soybean meal 44 % CP	932.92 ± 0.86	467.75 ± 2.63	14.13 ± 1.14	54.47 ± 1.79	73.47 ± 0.28	389.68 ± 6.61
Rapeseed meal	933.81 ± 0.72	407.60 ± 0.52	14.08 ± 3.02	79.87 ± 2.05	108.73 ± 2.26	389.12 ± 1.86
Full-fat soybean	947.85 ± 1.54	385.97 ± 1.23	181.99 ± 0.56	40.70 ± 0.00	60.61 ± 3.05	330.87 ± 5.57
Skim milk	970.13 ± 0.69	328.57 ± 3.45	10.98 ± 1.05	nd	75.77 ± 1.11	611.12 ± 0.56
Brewer's grain	943.47 ± 2.54	314.36 ± 4.33	63.64 ± 6.11	116.25 ± 2.65	37.90 ± 1.75	467.85 ± 5.82
Corn DDGS	919.02 ± 1.50	307.10 ± 0.74	102.03 ± 7.35	82.09 ± 1.67	55.97 ± 0.91	457.67 ± 5.03
Energy sources						
Broken rice	894.42 ± 3.56	95.19 ± 0.10	15.24 ± 3.32	14.22 ± 1.37	3.13 ± 0.24	872.22 ± 2.46
Corn	899.07 ± 2.39	90.02 ± 0.62	56.36 ± 2.19	17.16 ± 2.51	13.01 ± 1.17	823.46 ± 2.32
Rice bran	929.36 ± 1.47	161.36 ± 1.57	206.34 ± 3.67	92.31 ± 1.63	112.00 ± 0.92	425.86 ± 1.57
Coconut meal	934.97 ± 1.95	138.52 ± 3.29	204.19 ± 7.50	178.15 ± 5.75	41.81 ± 2.80	440.22 ± 7.16
Others						
Palm kernel meal	949.57 ± 3.35	168.01 ± 0.84	111.71 ± 1.93	181.18 ± 8.03	46.84 ± 2.24	495.61 ± 6.81
Soybean hull	925.90 ± 1.98	122.94 ± 0.67	33.84 ± 2.24	449.55 ± 5.73	45.20 ± 1.34	347.90 ± 6.10
Duckweed						
Lemna	914.79 ± 0.79	367.81 ± 3.58	52.47 ± 2.80	109.11 ± 7.18	142.06 ± 5.26	328.74 ± 0.66
Wolffia	953.21 ± 1.61	442.65 ± 1.90	16.74 ± 0.32	63.30 ± 2.82	149.34 ± 1.08	328.27 ± 5.56
Spirodela	935.41 ± 1.42	179.64 ± 0.15	48.23 ± 0.88	170.57 ± 0.30	212.40 ± 2.85	390.03 ± 1.44

nd = not detected; NFE = nitrogen free extract; Corn DDGS = corn distillers dries grains with soluble
Data are shown as the mean ± standard deviation.

The EE content was higher in rice bran (206.34 g/kg DM), coconut meal (204.19 g/kg DM) and full-fat soybean (181.99 g/kg DM) and lower in soybean meal (44 % CP; 14.13 g/kg DM), rapeseed meal (14.08 g/kg DM) and skim milk (10.98 g/kg DM). Meanwhile, EE content in duckweed was 52.47, 48.23 and 16.74 g/kg DM for *Lemna*, *Spirodela* and *Wolffia*, respectively. The ash content was observed higher in fishmeal (256.24 g/kg DM) and *Spirodela* (212.40 g/kg DM), whereas *Lemna* and *Wolffia* contained 142.06 and 149.06 g/kg DM, respectively. Furthermore, the NFE content was higher in broken rice (872.22 g/kg DM) and corn (823.46 g/kg DM), while there were 328.27, 328.74 and 390.03 g/kg DM for *Wolffia*, *Lemna* and *Spirodela*, respectively.

The nutritional values of all feed ingredients were within the range of tabulated values [30,31]. The chemical composition varied depending on type of feed ingredients and processing condition [32]. The animal products (skim milk, fishmeal and feather meal) had high CP content since protein is main compound in body composition [29]. In contrast, plant products contain high CF content as it forms a strong structure and is a highly variable component in plants [33]. Furthermore, the animal products were typically classified as protein sources due to their high CP with low CF contents. For plant products, protein sources included soybean meal (47 % CP), soybean meal (44 % CP), rapeseed meal, full-fat soybean, brewer's grain and corn DDGS. Furthermore, the energy sources included broken rice, corn, rice bran and coconut meal due to their low CP and low CF contents, but high NFE (starch and sugar) or EE (fat and lipid). The other groups included palm kernel meal and soybean hull, characterized by CF content exceeding 180.00 g/kg DM. In the case of duckweed, *Wolffia* and *Lemna* can be classified as protein sources due to their high

CP content (442.65 and 367.81 g/kg DM, respectively) and low CF content (63.30 and 109.11 g/kg DM, respectively). *Spirodela* was categorized as an energy source, with values of 179.64 g/kg DM for CP and 170.57 g/kg DM for CF. As shown in previous research [13,17,34,35], the chemical composition of duckweed was in a similar range, ranging from 184 to 439 g/kg DM for CP, and 122 to 250 g/kg DM for CF. Duckweed is capable of absorbing nutrients and nitrogen from the water resources in which it grows. However, the variation in the chemical composition and growth rate of duckweed is associated with several factors such as species, water quality, fertilizer application and cultivation condition [14,36].

In vitro **protein digestibility of duckweed and feed ingredients**

The true digestibility of duckweed and other feed ingredients are shown in **Figure 1**. The TD_{CP} of all feed ingredients ranged from 62 % for corn to 96 % for skim milk, whereas for duckweed, the values were 58, 75 and 79 % for *Spirodela*, *Wolffia* and *Lemna*, respectively. With regard to digestible CP content, the dTD_{CP} of all feed ingredients ranged from 55 g/kg DM for corn to 640 g/kg DM for feather meal, whereas for duckweed, the values were 105, 292 and 330 g/kg DM for *Spirodela*, *Lemna* and *Wolffia*, respectively. The SID_{CP} of duckweed and other feed ingredients are shown in **Figure 2**. The SID_{CP} ranged from 32 % for soybean hull to 96 % for skim milk, whereas for duckweed, the values were 39, 69 and 72 % for *Spirodela*, *Wolffia* and *Lemna*, respectively. The $dSID_{CP}$ of all feed ingredients ranged from 39 g/kg DM for soybean hull to 614 g/kg DM for feather meal, whereas for duckweed, the values were 70, 264 and 306 g/kg DM for *Spirodela*, *Lemna* and *Wolffia*, respectively.

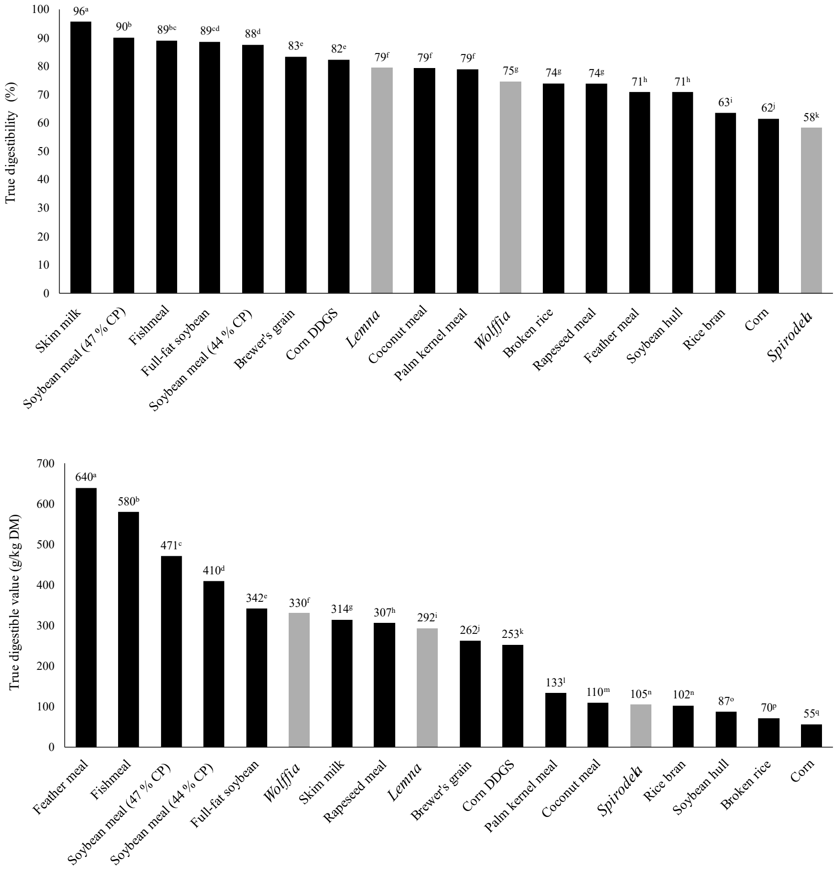

Figure 1 True digestibility (%) and true digestible value (g/kg DM) of duckweed and other feed ingredients Mean above a bar of each sample, superscripted with different lowercase letters are significantly ($p < 0.05$) different.

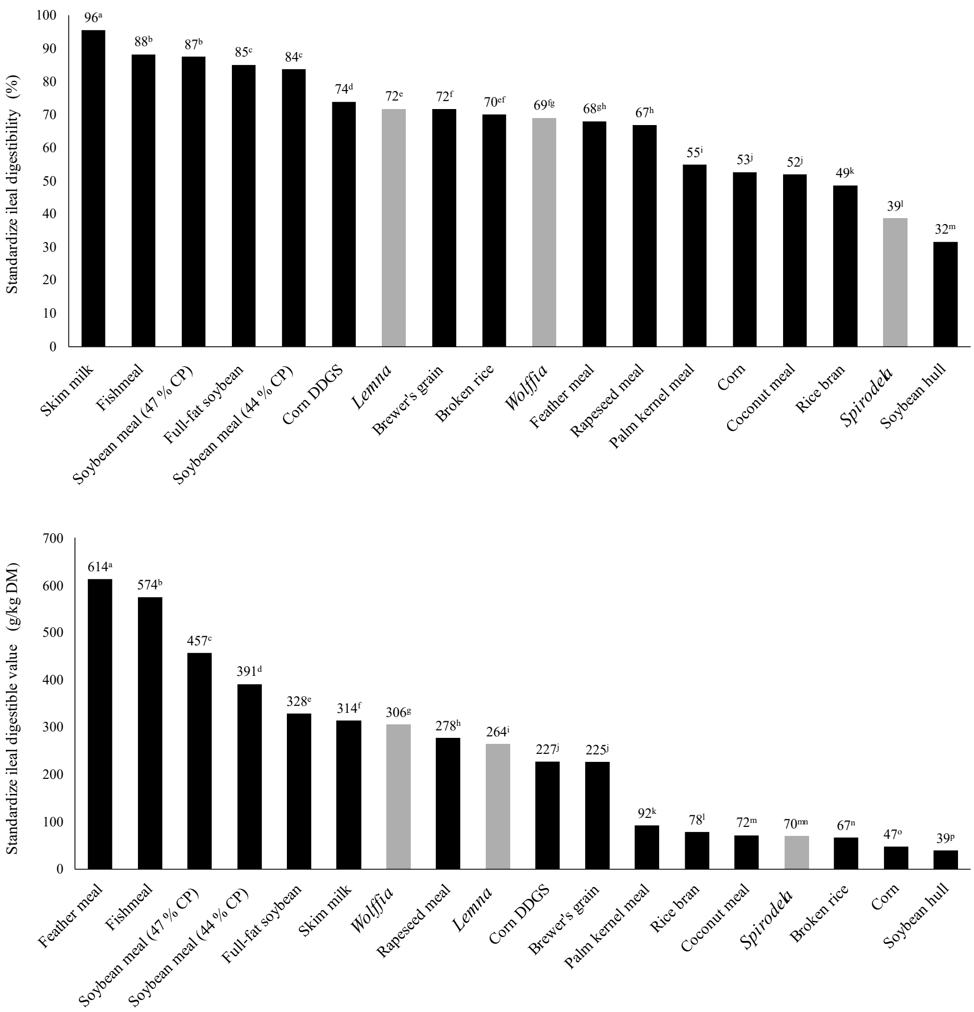

Figure 2 Standardize ileal digestibility (%) and standardized ileal digestible value (g/kg DM) of duckweed and other feed ingredients. Mean above a bar of each sample, superscripted with different lowercase letters are significantly ($p < 0.05$) different.

The TD_{CP} in soybean meal and rapeseed meal was within a range of previously published values [22], however, there are limited published data available for other feed ingredients. As TD_{CP} does not account for the endogenous protein loss from gastrointestinal tract, which significantly influence digestibility [37], SID_{CP} has been introduced to provide more accurate values derived from TD_{CP} by correcting for the endogenous protein loss [24,38]. The SID_{CP} values for all feed ingredients were consistent with values based on *in vivo* digestibility [39-41]. The highest SID_{CP} was observed in skim milk (96 %), indicating high protein quality. Skim milk is abundant in protein, consisting of 80 % casein and 20 % whey protein [42]. According to Mosenthin [43], diets containing casein were assumed to have 100 % ileal protein digestibility. For other animal protein sources, the SID_{CP} of fishmeal (88 %) was lower than that of skim milk. One possible explanation is that a large component of fishmeal is bone, which contains protein that is difficult to digest, resulting in a low SID_{CP} [44]. Furthermore, the SID_{CP} of feather meal (68 %) was lower than that of skim milk (96 %) and fishmeal (88 %), but consistent with a previous report by Sulabo *et al.* [45], ranging from 57.40 to 76.30 %. The use of feather meal in diets for monogastric animals is often limited due to the presence of keratin as the main component in feathers [32]. This protein is very rich in sulphur-containing amino acids with disulfide bonds, leading to a rigid structure [46] and low ileal digestibility of CP and amino acids, varying from 20 to 70 % [32].

With regard to plant-based ingredients, the SID_{CP} of soybean products (84 to 87 %) and rapeseed meal (67 %) were lower than that of skim milk and fishmeal. As more than 80 % of plant structure consists of cellulose and hemicellulose within a β-glycosidic linkage, this form is rigid and cannot digest by the enzymes of monogastric animals [47]. In addition, the higher fiber leads to increased secretion of mucin, epithelium cell and endogenous protein losses [48,49] as well as an increased passage rate and viscosity in the gastrointestinal tract [50], resulting in low protein digestibility [51]. This is in agreement with a previous study [52], which found that the amino acid digestibility of soybean decreased with increasing fiber content. Furthermore, in the present study, the low SID_{CP} was observed in soybean hull (32 %), coconut meal (52 %) and palm kernel meal (55 %) due to their high fiber content. Likewise, *Spirodela* exhibited the lowest SID_{CP} (39 %), which was lower than *Wolffia* (67 %) and *Lemna* (72 %), with *Spirodela* having the highest fiber (170.57 g/kg DM). Therefore, fiber content appears to be a primary factor limiting the nutrient digestibility of plant products.

An increase in ash content could reduce protein quality by decreasing the digestibility of amino acids [53]. Shirley and Parsons [54] indicated that the reduction in protein quality of meat and bone meal with increasing ash content. In the present study, the lower SID_{CP} of fishmeal compared to skim milk was attributed to its high ash content. Similarly, the lower SID_{CP} of rapeseed and rice bran than most feed ingredients were also due to their high ash content. For duckweed, the low SID_{CP} of *Spirodela* may be influenced by its high ash content (212.40 g/kg DM), which is higher than that of *Wolffia* (149.34 g/kg DM) and *Lemna* (142.06 g/kg DM).

Compared with feed table [55], the $dSID_{CP}$ values of soybean meal, fishmeal, corn DDGS, palm kernel meal and full-fat soybean were higher than the tabulated values, whereas the $dSID_{CP}$ values of feather meal, skim milk and corn were lower than the tabulated values. According to Kaewtapee *et al*. [56], the variations in $dSID_{CP}$ among feed ingredients are consistent with the differences in CP content and variation in SID_{CP}. For duckweed, *Wolffia* had higher $dSID_{CP}$ (306 g/kg DM) compared to *Lemna* (264 g/kg DM), despite having lower SID_{CP} (69 %) compared to *Lemna* (74 %). This difference can be attributed to the higher CP content in *Wolffia* (442.65 g/kg DM) compared to *Lemna* (367.81 g/kg DM). Conversely, *Spirodela* exhibited the lowest $dSID_{CP}$ due to its low CP content and SID_{CP}. Likewise, Demann *et al.* [17] reported that including *Spirodela polyrhiza* in the diet resulted in lower digestible CP compared to diets containing *Lemna obscura* due to the low CP content and digestibility in *Spirodela polyrhiza*. Notably, the CP content in duckweed obtained in the present study was higher than the values reported worldwide from the natural sources, ranging from 200 to 300 g/kg DM for *Wolffia* [34], and from 260 to 350 g/kg DM [18,57]. The possible explanation is that duckweed used in the present study was cultivated in a closed system with added fertilizers, whereas the others were grown in irrigation ponds or wastewater from pig farm with fewer nutrients. A previous study by Landolt [8] found that nitrate concentration in water resources had a significant impact on the CP content. Similarly, Petersen *et al*. [58] indicated that the CP content of *Lemna minor* and *Wolffiella hyalina* were improved due to increased ammonium concentration and a higher ammonium to nitrate ratio. Therefore, the CP content in duckweed varied depending on the nutrient concentration in the water resources and the cultivation conditions [59].

Hierarchical clustering of duckweed with all feed ingredients

The hierarchical clustering based on chemical composition and *in vitro* protein digestibility is showed in **Figure 3**, with all feed ingredients divided into 5 groups. The horizontal dendrogram displayed the distance or dissimilarity between clusters [60]. In this regard, fishmeal and feather meal were grouped together due to their similar NFE, dTD_{CP}, $dSID_{CP}$, CF and EE. Similarly, skim milk, soybean products and rapeseed were clustered in the same group owing to their similar TD_{CP}, SID_{CP}, CF and ash contents. Likewise, broken rice and corn were clustered together due to their almost similar nutritional profiles. For duckweed, *Lemna* and *Wolffia* were classified in the same group as brewer's grain and corn DDGS. This grouping may be attributed to their similar TD_{CP}, SID_{CP}, dTD_{CP}, CF and EE contents. Additionally, *Spirodela* was clustered with rice bran, palm kernel meal coconut meal and soybean hull due to their low contents of CP, dTD_{CP} and $dSID_{CP}$. The results suggested that *Lemna*, *Wolffia* and *Spirodela* can be considered as alternative feed ingredients based on their nutritional profiles within the same hierarchical clustering.

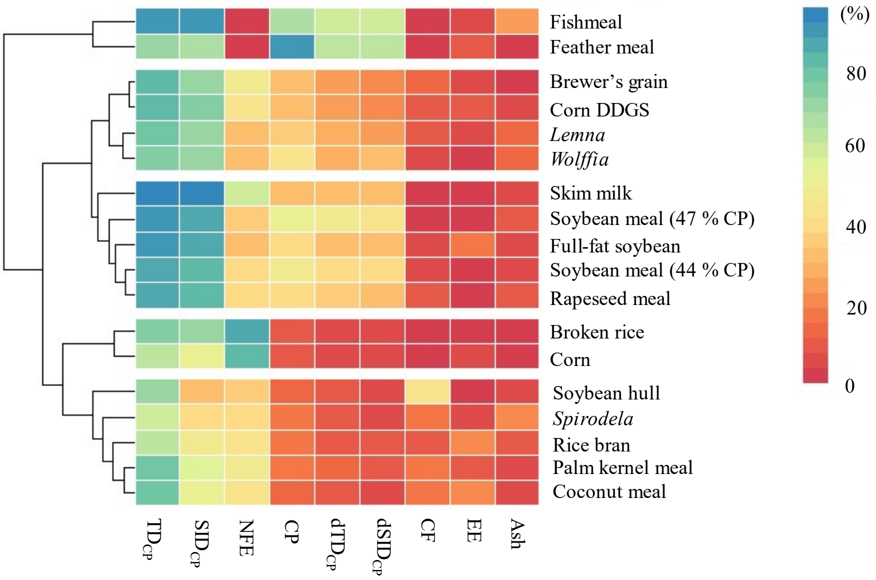

Figure 3 The correlation-based hierarchical clustering between duckweed and all feed ingredients; True digestibility of crude protein (TD_{CP}); Standardized ileal digestibility of crude protein (SID_{CP}); Nitrogen free extract (NFE); Crude protein (CP); True digestible value of crude protein (dTD_{CP}); Standardized ileal digestible crude protein ($dSID_{CP}$); Crude fiber (CF); Ether extract (EE).

Conclusions

Chemical composition and *in vitro* digestibility of duckweed varied depending on the different genera. *Wolffia* had a higher CP content compared to *Lemna* and *Spirodela*, whereas *Lemna* and *Spirodela* had a higher EE content than *Wolffia*. *Spirodela* had higher CF and ash contents, resulting in lower TD_{CP} and SID_{CP} compared to *Wolffia* and *Lemna* due to the limitation of enzymatic digestion in monogastric animals. However, the higher CP content in *Wolffia* resulted in greater dTD_{CP} and $dSID_{CP}$ when compared to corresponding values in *Lemna* and *Spirodela*. Based on nutritional values, *Lemna* and *Wolffia* were grouped with corn DDGS and brewer's grain, whereas *Spirodela* was grouped with soybean hull, rice bran, palm kernel meal and coconut meal. The results indicated that duckweed can be considered as a suitable alternative feed ingredients for sustainable livestock production.

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References

[1] GR Moekti. Industrial livestock production: A review on advantages and disadvantages. *IOP Conf. Ser. Earth Environ. Sci.* 2020; **492**, 012094.

[2] EU Ahiwe, AA Omede, MB Abdallh and PA Iji. *Managing dietary energy intake by broiler chickens to reduce production costs and improve product quality. In*: B Yucel and T Taskin (Eds.). Animal husbandry and nutrition. IntechOpen, London, 2018, p.115-45.

[3] G Fenwick. The assessment of a new protein source - rapeseed. *Proc. Nutr. Soc.*1982; **41**, 277-88.

[4] R Luthada-Raswiswi, S Mukaratirwa and G O'Brien. Animal protein sources as a substitute for fishmeal in aquaculture diets: A systematic review and meta-analysis. *Appl. Sci.* 2021; **11**, 3854.

[5] Y Zhang, M Luo, X Fang, F Zhang and M Cao. Energy value of rice, broken rice, and rice bran for broiler chickens by the regression method. *Poult. Sci*. 2021; **100**, 100972.

[6] O Onwudike. Palm kernel meal as a feed for poultry. 3. Replacement of groundnut cake by palm kernel meal in broiler diets. *Anim. Feed Sci. Tech*. 1986; **16**, 195-202.

[7] K Mat, Z Abdul Kari, ND Rusli, H Che Harun, LS Wei, MM Rahman, HN Mohd Khalid, MH Mohd Ali Hanafiah, SA Mohamad Sukri and RIA Raja Khalif. Coconut palm: Food, feed, and nutraceutical properties. *Animals* 2022; **12**, 2107.

[8] E Landolt. *The family of Lemnaceae-a monographic study*. Vol I. Veroeffentlichungen des Geobotanischen Institutes der ETH, Zurich, Switzerland, 1986.

[9] K Sree and KJ Appenroth. *Worldwide genetic resources of duckweed: Stock collections*. *In*: XH Cao, P Fourounjian and W Wang (Eds.). The duckweed genomes. Springer, Cham, Switzerland, 2020, p. 39-46.

[10] F Diwan. *Duckweed and its broad-spectrum applications.* Climate Survival Solutions, Colorado, 2023.

[11] X Ge, N Zhang, GC Phillips and J Xu. Growing *Lemna minor* in agricultural wastewater and converting the duckweed biomass to ethanol. *Bioresour. Tech*. 2012; **124**, 485-8.

[12] V Okomoda, S Solomon and G Ataguba. Potential uses of the family Lemnaceae. *J. Agr. Vet. Sci.* 2012; **4**, 1-14.

[13] LL Rusoff, EW Blakeney and DD Culley. Duckweeds (Lemnaceae family): A potential source of protein and amino acids. *J. Agr. Food Chem*. 1980; **28**, 848-50.

[14] M Sońta, A Rekiel and M Batorska. Use of duckweed (*Lemna* L.) in sustainable livestock production and aquaculture - a review. *Ann. Anim. Sci*. 2019; **19**, 257-71.

[15] N Shen, Q Wang, J Zhu, Y Qin, S Liao, Y Li, Q Zhu, Y Jin, L Du and R Huang. Succinic acid production from duckweed (*Landoltia punctata*) hydrolysate by batch fermentation of *Actinobacillus succinogenes* GXAS137. *Bioresour. Tech*. 2016; **211**, 307-12.

[16] GY Baek, M Saeed and HK Choi. Duckweeds: Their utilization, metabolites and cultivation. *Appl. Biol. Chem*. 2021; **64**, 73.

[17] J Demann, F Petersen, G Dusel, M Bog, R Devlamynck, A Ulbrich, HW Olfs and H Westendarp. Nutritional value of duckweed as protein feed for broiler chickens - digestibility of crude protein, amino acids and phosphorus. *Animals* 2023; **13**, 130.

[18] H Zakaria and M Shammout. Duckweed in irrigation water as a replacement of soybean meal in the laying hens' diet. *Braz. J. Poult. Sci*. 2018; **20**, 573-82.

[19] M Ahammad, M Swapon, T Yeasmin, M Rahman and M Ali. Replacement of sesame oil cake by duckweed (*Lemna minor*) in broiler diet. *Pak. J. Biol. Sci*. 2003; **6**, 1450-53.

[20] BS Moss. 1999, Economics and feed value of integrating duckweed production with a swine operation. Master Thesis. Texas Tech University, Texas, United States.

[21] OJ Rojas, Y Liu and HH Stein. Concentration of metabolizable energy and digestibility of energy, phosphorus, and amino acids in lemna protein concentrate fed to growing pigs. *J. Anim. Sci*. 2014; **92**, 5222-9.
[22] S Boisen and J Fernandez. Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by *in vitro* analyses. *Anim. Feed Sci. Tech.* 1995; **51**, 29-43.
[23] A Lemme, V Ravindran and WL Bryden. Ileal digestibility of amino acids in feed ingredients for broilers. *Worlds Poult. Sci. J.* 2004; **60**, 423-38.
[24] D Jezierny, R Mosenthin, N Sauer and M Eklund. *In vitro* prediction of standardised ileal crude protein and amino acid digestibilities in grain legumes for growing pigs. *Animals* 2010; **4**, 1987-96.
[25] NM de Carvalho, DL Oliveira, MAD Saleh, ME Pintado and AR Madureira. Importance of gastrointestinal *in vitro* models for the poultry industry and feed formulations. *Anim. Feed Sci. Technol.* 2021; **271**, 114730.
[26] AOAC International. *Association of official analytical collaboration*. AOAC International, Gaithersburg, 2005.
[27] R Core Team. *R: A language and environment for statistical computing, reference index version 4.3.1.* R Foundation for Statistical Computing, Vienna, Austria, 2023.
[28] G James, D Witten, T Hastie and R Tibshirani. *Classification*. *In*: G James, D Witten, T Hastie and R Tibshirani (Eds.). *An introduction to statistical learning.* 2nd ed. Springer, Cham, Switzerland, 2013, p. 129-95.
[29] GM Dryden. *Animal nutrition science*. Cabi, Wallingford, 2008.
[30] HH Stein, LV Lagos and GA Casas. Nutritional value of feed ingredients of plant origin fed to pigs. *Anim. Feed Sci. Tech*. 2016; **218**, 33-69.
[31] E Degussa. AMINODat® 5.0. Amino acid composition of feedstuffs. Evonik Degussa GmbH, Hanau, Germany, 2015.
[32] P McDonald, RA Edwards, JFD Greenhalgh, CA Morgan, LA Sinclair and RG Wilkinson. *Animal nutrition*. Trans-Atlantic, Philadelphia, 2011.
[33] B Kerr, W Dozier and G Shurson. Effects of reduced-oil corn distillers dried grains with solubles composition on digestible and metabolizable energy value and prediction in growing pigs. *J. Anim. Sci*. 2013; **91**, 3231-43.
[34] KJ Appenroth, KS Sree, M Bog, J Ecker, C Seeliger, V Böhm, S Lorkowski, K Sommer, W Vetter and K Tolzin-Banasch. Nutritional value of the duckweed species of the genus *Wolffia* (Lemnaceae) as human food. *Front. Chem*. 2018; **6**, 483.
[35] J Xu, Y Shen, Y Zheng, G Smith, XS Sun, D Wang, Y Zhao, W Zhang and Y Li. Duckweed (Lemnaceae) for potentially nutritious human food: A review. *Food Rev. Int*. 2023; **39**, 3620-34.
[36] I Showqi, FA Lone and JIA Bhat. Evaluation of the efficiency of duckweed (*Lemna minor* L.) as a phytoremediation agent in wastewater treatment in Kashmir Himalayas. *J. Biorem. Biodegrad.* 2017; **8**, 405.
[37] S Boisen and BO Eggum. Critical evaluation of *in vitro* methods for estimating digestibility in simple-stomach animals. *Nutr. Res. Rev.* 1991; **4**, 141-62.
[38] S Boisen. *In vitro* analyses for predicting standardised ileal digestibility of protein and amino acids in actual batches of feedstuffs and diets for pigs. *Livest. Sci.* 2007; **109**, 182-5.
[39] DJ Bloxham, M Azain, GM Pesti and SB Wu. Total amino acid level affects the results of standardized ileal digestibility assays for feed ingredients for swine. *Anim. Nutr.* 2023; **13**, 19-30.

[40] C Pedersen and S Boisen. Establishment of tabulated values for standardized ileal digestibility of crude protein and essential amino acids in common feedstuffs for pigs. *Acta Agr. Scand. Sect. A Anim. Sci*. 2002; **52**, 121-40.

[41] YL Yin, TJ Li, RL Huang, ZQ Liu, XF Kong, WY Chu and FG Yin. Evaluating standardized ileal digestibility of amino acids in growing pigs. *Anim. Feed Sci. Tech*. 2008; **140**, 385-401.

[42] N Fu, S Huang, J Xiao and XD Chen. Producing powders containing active dry probiotics with the aid of spray drying. *Adv. Food Nutr. Res.* 2018; **85**, 211-62.

[43] R Mosenthin. Current concepts of protein digestion and absorption in the pig review. *Vet. Zootech*. 2003; **20**, 30-8.

[44] O Rojas and HH Stein. Concentration of digestible, metabolizable, and net energy and digestibility of energy and nutrients in fermented soybean meal, conventional soybean meal, and fish meal fed to weanling pigs. *J. Anim. Sci*. 2013; **91**, 4397-405.

[45] RC Sulabo, LI Chiba, FN Almeida, SD Brotzge, RL Payne and HH Stein. Amino acid and phosphorus digestibility and concentration of digestible and metabolizable energy in hydrolyzed feather meal fed to growing pigs. *J. Anim. Sci.* 2013; **91**, 5829-37.

[46] Y Zhang, R Yang and W Zhao. Improving digestibility of feather meal by steam flash explosion. *J. Agric. Food Chem*. 2014; **62**, 2745-51.

[47] BJ Kerr and GC Shurson. Strategies to improve fiber utilization in swine. *J. Anim. Sci. Biotechnol*. 2013; **4**, 11.

[48] C Parsons, L Potter and R Brown. Effects of dietary carbohydrate and of intestinal microflora on excretion of endogenous amino acids by poultry. *Poult. Sci.* 1983; **62**, 483-9.

[49] R Mosenthin and W Sauer. The effect of source of fiber on pancreatic secretions and on amino acid digestibility in the pig. *J. Anim. Physiol. Anim. Nutr.* 1991; **65**, 45-52.

[50] ZJ Sandor, N Revesz, D Varga, F Toth, L Ardo and G Gyalog. Nutritional and economic benefits of using DDGS (distiller'dried grains soluble) as feed ingredient in common carp semi-intensive pond culture. *Aquacult. Rep*. 2021; **21**, 100819.

[51] L Montagne, J Pluske and DA Hampson. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Anim. Feed Sci. Tech*. 2003; **108**, 95-117.

[52] R Mosenthin, WC Sauer and F Ahrens. Dietary pectin's effect on ileal and fecal amino acid digestibility and exocrine pancreatic secretions in growing pigs. *J. Nutr*. 1994; **124**, 1222-9.

[53] X Wang and CM Parsons. Effect of raw material source, processing system, and processing temperatures on amino acid digestibility of meat and bone meals. *Poult. Sci*. 1998; **77**, 834-41.

[54] R Shirley and C Parsons. Effect of ash content on protein quality of meat and bone meal. *Poult. Sci*. 2001; **80**, 626-32.

[55] National Research Council. *Nutrient requirements of swine*. 11th ed. National Academy Press, Washington DC, 2012.

[56] C Kaewtapee, R Mosenthin, S Nenning, M Schäffler, M Eklund and P Rosenfelder-Kuon. Standardized ileal digestibility of amino acids in European soya bean and rapeseed products fed to growing pigs. *J. Anim. Physiol. Anim. Nutr*. 2017; **102**, e695-e705.

[57] RA Mohedano, RHR Costa, FA Tavares and PB Filho. High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds. *Bioresour. Tech*. 2012; **112**, 98-104.

[58] F Petersen, J Demann, D Restemeyer, A Ulbrich, HW Olfs, H Westendarp and KJ Appenroth. Influence of the nitrate-n to ammonium-n ratio on relative growth rate and crude protein content in the duckweeds *Lemna minor* and *Wolffiella hyalina*. *Plants* 2021; **10**, 1741.

[59] J Xu, W Cui, JJ Cheng and AM Stomp. Production of high-starch duckweed and its conversion to bioethanol. *Biosyst. Eng.* 2011; **110**, 67-72.

[60] J Phipps. Dendrogram topology. *Syst. Zool.* 1971; **20**, 306-8.